

NOTES ON THE BUMBLE-BEE
(*BOMBUS FERVIDUS FABRICIUS*)
AND ITS CHROMOSOMES

By ROY M. WHELDEN

UNION COLLEGE, SCHENECTADY, NEW YORK

One of the more common of the bumble-bees in northern New England is *B. fervidus*. Yet its nests are not always easily found and collected. So when, in the present case, a nest was located in the open space beneath the frames of an abandoned beehive, collection seemed imperative, especially as it was necessary to move the hive.

The nest was a loose mass of soft, lightly entangled, slender grass blades, amongst which there were a few bits of goose-down. The position of the beehive made it almost certain that all this material was carried in and arranged by the bees. In the center of this mass, there was the single irregular comb, about 12cm. across, resting on a thin layer of grass blades. Collection was made late in the afternoon of August 19, 1947, and included all foraging bees as they returned (from foraging). Presumably the entire colony was taken: no additional bees were observed later.

Fixation was in a modification of Bouin's solution, the adults being plunged directly into this fluid without dissection. The contents of the cells were removed carefully and also put into the fixing fluid. Finally several pieces of the comb itself were dropped into the fixative. Five separate cells in the comb were partially full of a very thick dark brown honey which was extremely fragrant and of a very fine taste.

The nest contained four eggs, fifteen larvæ, forty-two pupæ (plus one male prepupa) and seventeen adults. Later on, each of these was sectioned and stained in Heidenhain's hæmatoxylin, as were a few of the emptied cells. The adult bees comprised one conspicuously large specimen, 21.5 mm. long, and an extremely small one (scarcely 7.8 mm. long; all the others were very uniformly 13 — 16 mm.

long). Of this group, ten were very definitely worker adults, containing very small undeveloped ovaries with no eggs; the remaining five were male bees, with mature sperm.

Of the pupæ, 37 were found to be males, five were apparently to become workers, and one only was a queen.

The larvæ fall into three very distinct size groups; seven are large ones, nearly fully grown. Of these, six are males, the other probably a worker. Two of the males are about half the length of the preceding group. Six larvæ are very small; two of these are definitely males, the other four are as yet indeterminate.

One of the eggs was newly laid and uninucleate. The others all had embryo developed to cover the greater part of the yolk surface. In one, development of the stomo- and proctodæum was well advanced, but differentiation was not great. Two of the eggs showed a great many cells with nuclei in various stages of division. Each nucleus contained a single rather prominent nucleolus, and an indeterminate number of very small uniformly dispersed chromatin granules. The surrounding cytoplasm was noticeably more dense than that of the nucleus and quite uniform. The earliest evidence of impending division was found in a gradual increase in the amount of dark staining material, which tended gradually to aggregate to form what at first appeared to be a continuous but irregular filament, and then with increasing diameter broke into discrete elongate particles. There were twelve of these; usually it was difficult to determine the exact number at this stage, since they were not all in one plane. (Fig. 1.) Typical division stages showed the various phases passed through, (Fig. 2 & 3 for anaphase and Fig. 6 for metaphase and telophase). There was one conspicuous exception to this series, and it was shown quite clearly in a great many cells in these eggs. Mostly these cells were in the epidermis, and were usually rather conspicuous because they protruded above the general level of the egg surface, often for about one half their diameter. In these cells, the number of chromosomes was just double that of those above described, being clearly twenty-four. Two of these cells are shown in metaphase in Figs. 4 & 5. It is to be noted that these cells seem to be rather uniformly distributed in the epidermal tissues.

Dividing nuclei occurred rather sparingly in the cells of the fifteen larvæ, and showed little that was not seen earlier in the eggs. Here, however, there was greater difference in the sizes of the cells, with corresponding but smaller differences in the nuclear sizes. In Figure 7 is shown a typical epidermal cell, with the twelve chromosomes forming a compact group in a faintly staining enveloping substance. Surrounding this there is a conspicuously clear zone, in turn surrounded by the darker mass of uniformly fine granular cytoplasm. Figure 8 presents a chromosome picture that is frequently seen in this bee, that of two chromosomes noticeably smaller than the other ten. This apparently is not so in all the cells, as is clear from a comparison of these two figures.

In the larvæ were to be seen frequently cells in which there were more than the usual number of chromosomes, usually 24 instead of 12. (Fig. 10.) As in the eggs, these cells occurred singly, almost always in the epidermis. Occasionally a cell was noted which seemed to have even more than 24 chromosomes. Figure 9 is an example, in which there appear to be at least thirty chromosomes. No differences were to be found between corresponding cells in worker and male larvæ. No good dividing nuclei were found in the testes in larvæ.

Among the pupæ, only in the males were there cells showing good nuclear divisions. In them these were often abundant, especially in the testes. Some of the individuals showed no indication of reduction divisions, the testes still being formed of uniformly regular crowded cells (Fig. 11); other specimens showed testes in which sperm maturation was nearly completed. In individuals between these two groups, there were often large numbers of dividing cells in all stages, from regular divisions of undifferentiated cells showing the usual twelve quite uniform chromosomes (Figs. 12-15), to those showing various stages in reduction division (Figs. 18-23). The latter occurred in scattered groups in the several testes lobes, each group seeming to be very uniform in the stage and rate of division of its cells; but among the different groups, there was the greatest of irregularity.

One fact was very frequently noticeable — when the chromosomes began moving from the metaphase plate to the poles, often very

great irregularity seemed to be the rule. Another noticeable feature was the presence of one or two small granules among the chromosomes. Perhaps there were always two of these granules, but in many cases, there seemed to be only one. Not infrequently none was observed, especially in the earlier stages of division. It is easy to suppose that even if present one or both of these granules could be obscured by a much larger chromosome. With separation of the chromosome halves and migration to the spindle poles, these granules seemed to be much more frequently observed, when they were quite often seen lagging behind on the spindle, even after the chromosomes had begun to lose their identity when merging into a forming nucleus. (Figs. 15, 17, 21 & 22). In no case could any connection of the granules to any chromosome be detected.

At the completion of the reduction divisions, there were often formed rather large spherical cells not unlike the cells forming the early testes except that these following reductions had very small spherical nuclei about 2μ in diameter. (Fig. 26.) In other and apparently more usual cases, reduction division led into sperm maturation at once. Well before the end of pupation, sperm formation had been completed in the entire testes.

In the pupa, many cells were observed to have a larger chromosome number than twelve. Most conspicuous of these polyploid cells were some in the forepart of the intestine, where the cells occurred singly, were of noticeably larger size and when properly oriented showed conspicuous spindles. (Fig. 28.) In these cells the chromosome number was frequently 24, but occasionally appeared to be at least 30 or higher. (Fig. 27.) In the forepart of the gut, in the region of the proventriculus, similar large cells with increased chromosome numbers were often observed. (Fig. 25.)

In the head, where they were apparently always limited to certain of the appendages and to the epidermis, there were some exceptionally large cells. No satisfactory divisions were observed in any of these cells, but a few gave indication that the chromosome numbers were large, perhaps as high as 36.

As is usual in Hymenoptera, the cells of the brain cortex showed a very great range in size, some being truly gigantic. In no case

could an accurate chromosome count be made in any of these large cells, but it was definitely in the order of 24 and 36.

Little need be said of the adults here. The only dividing nuclei found in any of them were in the queens, and there only in the cells of the follicular epithelium. Even here nuclear divisions were rarely found. In these, there were invariably twelve chromosomes, very small here as would be expected, considering the small size of the cells.

Presumably such reduction divisions as were to occur in the females had been completed before fixation. But all stages in egg formation were found. In the smallest eggs, scarcely distinct from the surrounding cells, the nucleus was relatively very large, with conspicuously sparse chromatin substance. As egg enlargement progressed, the egg nucleus gradually changed its position and its shape, moving to a position near the lower end of the egg, until it seemed pressed against the egg membrane and had a thick discoid shape. Later in development, it moved away from this position and once again became spherical.

Sectioning of the cells of the comb showed that in nearly all cases the inner wall of the cell was covered with an irregular layer of pollen grains, sometimes sparse and sometimes two to four grains thick. These grains were from four different plants, in about equal numbers. Very rarely a grain of some other plant was seen. These were of the same species as those which were observed in the mid-guts of the several larvæ.

EXPLANATION OF FIGURES

1. Epidermal cell of embryo in egg, the chromatin now aggregated into twelve chromosomes.
2. From the same egg, an epidermal cell in early anaphase, the twelve pairs of chromosomes being quite clear. The darker ones are above the shaded ones.
3. Two epidermal cells, one showing a later stage of anaphase. The nucleus of the cell at the right is sectioned, only $\frac{1}{2}$ being figured in this section. The chorion is indicated above the cells.
4. Two complete epidermal cells plus a portion of a third from the epidermal layer of the same egg. One cell protrudes very prominently above the surface of the egg; in this there are 24 chromosomes.
5. Another of these protruding cells, also with 24 chromosomes, from another egg.
6. Two cells from stomodaeum, that at the left with twelve chromosomes at metaphase, that at the right in (late) telophase, the nuclear division nearing completion. These cells border the lumen of the stomodaeum.
7. Surface cell from a small worker larva, showing the twelve small chromosomes.
8. Cell from a medium sized worker larva, showing twelve chromosomes, two of which are conspicuously smaller.
9. Epidermal cell of same medium sized larva, with over 30 chromosomes.
10. Surface cell of medium sized larva, showing one of the many cells having a greater number of chromosomes. In this nucleus there are 24, in metaphase.
11. Spermatogonia, in early prophase.
12. & 13. Spermatogonia, showing metaphase plate, with twelve chromosomes, plus small granule.
14. Spermatogonium, showing 12 chromosomes, plus very small granule, very irregularly distributed on the spindle.
15. Spermatogonium, in mid- anaphase, with twelve chromosomes to each half, plus a minute granule.
16. Spermatogonium, showing bent spindle.
17. Early telophase, with minute granule remaining remote from two chromosome masses.
18. Stage of second spermatocyte division.
19. Early pairing of chromosomes in spermatocyte division.
20. Spermatocyte division, showing six chromosomes, plus two minute particles.
21. Late anaphase, showing six pairs of chromosomes, plus two small granules, in spindle fibres.
22. Group of three cells in early to mid- telophase, showing lagging particles; and at right, six chromosomes at each pole.
23. A cell similar to one at right above, but cut transversely, showing the six small chromosomes rather clearly.
24. In wall of oesophagus, in thorax of male pupa, showing late anaphase with twelve chromosomes, plus small granule.
25. In same region as fig. 24, but in metaphase, and with 24 chromosomes.
26. Cell before beginning of sperm formation.
27. Large cell in fore part of intestine, showing at least thirty chromosomes.
28. Cell in same region, at right angles to that in fig. 27.

